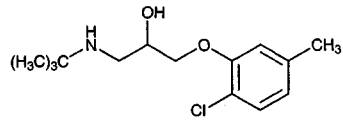
Bupranolol



Molecular formula: $C_{14}H_{22}ClNO_2$
Molecular weight: 271.79
CAS Registry No.: 14556-46-8, 15148-80-8 (HCl)
Merck Index: 1521

SAMPLE

Matrix: solutions
Sample preparation: Mix 300 µL of a solution in chloroform with 20 µL 0.1% (+)-(S)-naphthylethylisocyanate in chloroform. Mix, let stand at room temperature for 20 min. Evaporate to dryness. Redissolve the residue in 300 µL mobile phase. Inject a 50 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Partisil 5 silica (Phenomenex)
Mobile phase: Hexane:chloroform:MeOH 74:25:1
Flow rate: 1
Injection volume: 50
Detector: UV 282

CHROMATOGRAM

Retention time: 8.1, 10.6 (enantiomers)

OTHER SUBSTANCES

Simultaneous: mefloquine

KEY WORDS

chiral; derivatization; siliconize glassware; normal phase; normal phase is superior to reverse-phase procedure

REFERENCE

Souri,E.; Farsam,H.; Jamali,F. Stereospecific determination of mefloquine in biological fluids by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *700*, 215–222.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 µm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 1.85

OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE

Matrix: solutions

Sample preparation: Mix 100 μL of a 10 μM solution in MeCN:water:triethylamine 50:50:0.1 with 100 μL 1 mM (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in MeCN, heat in the dark at 65° for 1.5 h, inject an aliquot. (Synthesis of (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene from EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ TLC plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane:acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the mini-

mum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei. Add 100 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL 5% HCl, wash 3 times with 50 mL portions of ethyl acetate, adjust the pH of the aqueous solution to 13-14 with 5% NaOH, extract 6 times with 50 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as orange crystals (mp 96-98°) (Analyst 1992, 117, 727). Add 100 μL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25 mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as yellow crystals (mp 160-170° d) (Analyst 1995, 120, 385).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-80A
Mobile phase: MeCN:water:trifluoroacetic acid 50:50:0.1
Column temperature: 40
Flow rate: 1
Detector: F ex 460 em 550

CHROMATOGRAM

Retention time: 29.4, 36.5 (enantiomers)
Limit of detection: 0.00125-0.00161 fmole

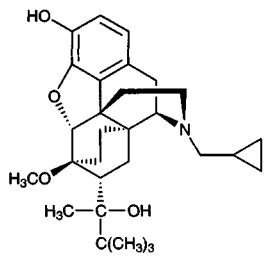
KEY WORDS

derivatization; chiral

REFERENCE

Toyo'oka,T.; Toriumi,M.; Ishii,Y. Enantioseparation of β-blockers labelled with a chiral fluorescent reagent, R(-)-DBD-PyNCS, by reversed-phase liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1467–1476.

Buprenorphine



Molecular formula: $C_{29}H_{41}NO_4$
Molecular weight: 467.65
CAS Registry No.: 52485-79-7, 53152-21-9 (HCl)
Merck Index: 1522
Lednicer No.: 2 321

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 100 µL 500 ng/mL IS in MeOH + 100 µL 1 M NaOH, mix, extract with 6 mL hexane:isopropanol 99:1 by rotary mixing for 10 min, centrifuge at 2000 g for 5 min. Add 300 µL 200 mM HCl to the organic layer, mix, centrifuge at 2000 g for 5 min, discard the upper organic layer, inject a 120 µL aliquot of the acidic aqueous phase.

HPLC VARIABLES

Column: 150 × 4.6 Spherisorb C8
Mobile phase: MeCN:triethylamine:Pic B5:60 mM pH 6.4 phosphate buffer 48:0.05:0.15:52, adjusted to pH 6.4 with orthophosphoric acid
Flow rate: 1.6
Injection volume: 120
Detector: UV 214

CHROMATOGRAM

Retention time: 5.2
Internal standard: clothiapine (9.6)
Limit of detection: 1 ng/mL
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Simultaneous: amitriptyline, amoxapine, carpipramine, clomipramine, demexiptilline, desipramine, diazepam, imipramine, medifoxamine, metapramine, methadone, mianserine, naloxone, normaprotiline, opipramol, paroxetine, quinupramine, tianeptine, trazodone, viloxazine
Noninterfering: codeine, codethyline, morphine, pholcodeine
Interfering: dosulepin, doxepin, maprotiline, nortriptyline, oxaflozane

KEY WORDS

plasma

REFERENCE

Lagrange,F.; Pehourcq,F.; Baumvieille,M.; Begaud,B. Determination of buprenorphine in plasma by liquid chromatography: application to heroin-dependent subjects, *J.Pharm.Biomed.Anal.*, **1998**, *16*, 1295–1300.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 50 µL 5 µg/mL nalbuphine in water + 1 mL 500 mM pH 9.25 sodium carbonate buffer + 3 mL hexane:isoamyl alcohol 9:1, mix on a rotary shaker for 30 min, centrifuge at 1880 g for 20 min, freeze at -20° for 1 h (for rabbit plasma perform on half-scale). Remove the organic layer and evaporate it to dryness under a stream of air, reconstitute in 250 µL mobile phase, inject a 200 µL aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm Applied Biosystems pre-column
Column: 100 × 2 10 μm μPorasil
Mobile phase: MeCN:5 mM pH 3.75 sodium acetate 80:20
Flow rate: 1
Injection volume: 200
Detector: F ex 210 em 345

CHROMATOGRAM

Retention time: 8.83
Internal standard: nalbuphine (11.7)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Simultaneous: butorphanol, morphine, ethylmorphine, codeine, nalorphine, fentanyl, meperidine, tramadol
Noninterfering: thiopentone, succinylcholine, pancuronium, diazepam, atropine, neostigmine

KEY WORDS

plasma; human; pig; dog; rabbit; pharmacokinetics

REFERENCE

Ho,S.-T.; Wang,J.-J.; Ho,W.; Hu,O.Y.-P. Determination of buprenorphine by high-performance liquid chromatography with fluorescence detection: application to human and rabbit pharmacokinetic studies, *J.Chromatogr.*, **1991**, *570*, 339–350.

SAMPLE

Matrix: blood
Sample preparation: Rock 5 mL whole blood + 10 mL water + 8.5 mL Na_2WO_4 in a 50 mL stoppered tube for 1 min, add 6 mL $NiCl_2$, rock for 5 min, add 15 mL dichloromethane: isobutyl alcohol:THF 30:45:25, centrifuge at 2500 g for 15 min. Remove organic phase and repeat the process. Filter all organic phases through a 40-90 μm filter and evaporate to dryness in a 100 mL porcelain dish at a moderate temperature in a sand bath. Take up residue in 500 μL MeCN:water 80:20, inject a 20 μL aliquot. (Na_2WO_4 prepared by mixing 10 g $Na_2WO_4.2H_2O$ in 38 mL of 2 M NaOH and 2.5 g of $NaHCO_3$ and making up to 100 mL. $NiCl_2$ was 17% w/v $NiCl_2$ in water.)

HPLC VARIABLES

Column: 200 × 4.6 5 μm Hypersil C8
Mobile phase: A = MeCN; B = 20 mM n-propylamine adjusted to pH 5 with 85% phosphoric acid. A:B from 15:85 to 20:80 over 5 min to 45:55 over another 15 min to 65:35 over another 5 min
Injection volume: 20
Detector: UV 230

CHROMATOGRAM

Retention time: 26
Limit of detection: 0.30 ppm

OTHER SUBSTANCES

Extracted: caffeine, cocaine, codeine, diamorphine, ethylmorphine, lidocaine, methaqualone, morphine, naloxone, noscapine, papaverine, pentazocine, procaine
Also analyzed: bromazepam, clonazepam, diazepam, flunitrazepam, flurazepam, medazepam, nitrazepam, oxazepam

KEY WORDS

whole blood

REFERENCE

Bernal,J.L.; Del Nozal,M.J.; Rosas,V.; Villarino,A. Extraction of basic drugs from whole blood and determination by high performance liquid chromatography, *Chromatographia*, **1994**, *38*, 617–623.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 288

CHROMATOGRAM

Retention time: 5.36

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen;

tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, hair, urine

Sample preparation: Blood, urine. Mix 2 mL whole blood, plasma, or urine with 10 ng IS, 1.5 mL saturated pH 8.4 ammonium hydrogen phosphate buffer, and 5 mL chloroform: isopropanol:n-heptane 25:10:65. (Caution! Chloroform is a carcinogen!) Agitate, centrifuge at 3500 g for 10 min, evaporate the organic layer at 45° for 30 min (Speed Vac Concentrator). Reconstitute the dry extract in 20 μL mobile phase, centrifuge at 10000 g for 5 min, inject a 2 μL aliquot of the supernatant. Hair. Decontaminate hair with two portions of dichloromethane for 2 min, pulverize for 5 to 10 min (Retsch MM2 ball mill). Mix 40 mg powdered hair with 1 ng IS and incubate in 1 mL 100 mM HCl overnight at 56°. Neutralize 1 mL hair homogenate using 1 mL 100 mM NaOH, add 1.5 mL saturated pH 8.4 ammonium hydrogen phosphate, extract with 5 mL chloroform:isopropanol:n-heptane 25:10:65. Agitate mixture, centrifuge at 3500 g for 10 min, evaporate the organic layer at 45° for 30 min (Speed Vac Concentrator). Reconstitute the dry extract in 20 μL mobile phase, centrifuge at 10000 g for 5 min, inject a 2 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 15 × 1.0 5 μm Opti-Guard C18 (Interchim)
Column: 150 × 2.0 4 μm NovaPak C18 (Waters)
Mobile phase: MeCN:2 mM pH 3.0 ammonium acetate buffer 80:20
Flow rate: 0.2
Injection volume: 2
Detector: MS, Perkin Elmer Sciex API-100, ionspray interface, a post column split 1:3 reduces the flow rate to 50 μL/min for infusion into HPLC/MS interface, nebulizing gas nitrogen (99.95%, 40 psi, FR 1.16 L/min) and curtain gas (FR 1.08 L/min). Ion sampling at +50 V, electron multiplier +2400 V, mass range m/z 260-475, MIM at m/z 414 (norbuprenorphine), 468 (buprenorphine), and 472 (buprenorphine-d4)

CHROMATOGRAM

Retention time: 5.84
Internal standard: buprenorphine-d4 (Radian) (5.79)
Limit of detection: 100 pg/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; whole blood

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. HPLC/MS determination of buprenorphine and norbuprenorphine in biological fluids and hair samples, *J.Forensic Sci.*, **1997**, *42*, 111–114.

SAMPLE

Matrix: blood, urine

Sample preparation: Condition a 1 mL 40 μm Supelclean SPE cartridge with 4 mL MeOH and 4 mL water. Add 5 mL serum, plasma, or urine to the SPE cartridge, add 400 μL 50 mM borate buffer adjusted to pH 9.1 with 1 M NaOH, wash with 600 μL MeCN:50 mM NaH_2PO_4 20:80, elute slowly with 3 mL chloroform. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 500 μL 50 mM NaH_2PO_4 in MeCN:water 50:50 (pH adjusted to 2.5 with phosphoric acid) by shaking at 70° for 20 min, dilute with 3 mL water, add 500 μL 50 mM borate buffer adjusted to pH 9.1 with 1 M NaOH, inject a 2 mL sample onto column A with mobile phase A, after 2 min backflush the contents of column A onto column B with mobile phase B, elute column B with mobile phase B for 8 min and monitor the effluent. At the end of the process backflush column B with mobile phase B for 8 min.

HPLC VARIABLES

Column: A 4 × 4 5 μm RP-18 (E. Merck); B 30 × 4 5 μm C18 (Macherey & Nagel)
Mobile phase: A MeCN:50 mM phosphate buffer adjusted to pH 8.5 with 1 M NaOH 20:80 (Use a 250 × 4 column of 40 μm silica between pump and injector to protect column A.); B MeCN:50 mM NaH_2PO_4 25:75
Flow rate: A 2; B 0.8
Injection volume: 2000
Detector: E, ESA Coulochem 5100 A detector, Model 5020 guard cell 500 mV, Model 5010 analytical cell, detector 1 160 mV, detector 2 480 mV, monitor detector 2

CHROMATOGRAM

Retention time: 5
Limit of detection: 0.04 ng/mL

KEY WORDS

serum; plasma; SPE; column-switching; pharmacokinetics

REFERENCE

Schleyer,E.; Lohmann,R.; Rolf,C.; Gralow,A.; Kaufmann,C.C.; Unterhalt,M.; Hiddemann,W. Column-switching solid-phase trace-enrichment high-performance liquid chromatographic method for measurement of buprenorphine and norbuprenorphine in human plasma and urine by electrochemical detection, *J.Chromatogr.*, **1993**, *614*, 275–283.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 212.2

CHROMATOGRAM
Retention time: 14.035

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 300 mm long C18
Mobile phase: MeCN:5 mM heptanesulfonic acid adjusted to pH 3.5 with glacial acetic acid 35:65
Injection volume: 20
Detector: UV 212

CHROMATOGRAM
Limit of quantitation: 80 ng/mL

KEY WORDS
nasal solutions

REFERENCE
Gries,W.J.; Wan,W.; Matos,F.J.; de Meireles,J.C.; Pimplaskar,H.K.; Sileno,A.P.; Romeo,V.D.; Xia,W.J.; Behl,C.R. A specific and sensitive method for quantitating buprenorphine hydrochloride in a nasal solution (Abstract 2517), *Pharm.Res.*, **1997**, *14*, S381–S381.

SAMPLE
Matrix: hair
Sample preparation: Wash 50 mg hair twice with 5 mL dichloromethane for 2 min, pulverize in a ball mill, incubate in 1 mL 100 mM HCl overnight at 56°, neutralize with 1 mL 100 mM NaOH, add 1 mL pH 8.5 phosphate buffer, extract with 5 mL toluene, agitate, centrifuge. Remove the organic phase and extract it with 1 mL 100 mM HCl. Remove the aqueous phase and add it to 1 mL 100 mM NaOH, 1 mL pH 8.5 phosphate buffer, ann 5 mL toluene. Agitate, centrifuge, remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 60 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Lichrosorb CN
Mobile phase: MeCN:10 mM pH 4.0 phosphate buffer:1-heptanesulfonic acid:butylamine 17:85:2:0.01
Flow rate: 1
Injection volume: 60
Detector: E, ESA Coulochem II, first electrode +150 mV, second electrode +500 mV

CHROMATOGRAM
Retention time: 10.71
Limit of detection: 20 ng/g

OTHER SUBSTANCES
Extracted: norbuprenorphine

REFERENCE

Kintz,P. Determination of buprenorphine and its dealkylated metabolite in human hair, *J.Anal.Toxicol.*, **1993**, *17*, 443–444.

SAMPLE

Matrix: hair

Sample preparation: Wash 50 mg hair with 5 mL dichloromethane for 2 min, repeat wash, pulverize in a ball mill, add 1 mL 100 mM HCl, heat at 56° overnight, neutralize, add 1 mL pH 8.5 saturated phosphate buffer, add 10 mL toluene, agitate, centrifuge. Remove the organic layer and add it to 5 mL 100 mM HCl, extract. Remove the aqueous layer and add it to 1 mL ammonia solution, add 1 mL ph 8.5 phosphate buffer, add 5 mL toluene, agitate, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 60 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 5 μm Lichrosorb CN
Mobile phase: MeCN:10 mM pH 4.0 phosphate buffer:1-heptanesulfonic acid 85:17:0.01 (?)
Flow rate: 1
Injection volume: 60
Detector: E, ESA Coulochem II, first electrode +0.15 V, second electrode +0.50 V (monitored)

CHROMATOGRAM

Limit of detection: 0.02 ng/g

OTHER SUBSTANCES

Extracted: metabolites

REFERENCE

Kintz,P.; Cirimele,V.; Edel,Y.; Jamey,C.; Mangin,P. Hair analysis for buprenorphine and its dealkylated metabolite by RIA and confirmation by LC/ECD, *J.Forensic Sci.*, **1994**, *39*, 1497–1503.

SAMPLE

Matrix: perfusate

HPLC VARIABLES

Column: μBondapak C18
Mobile phase: MeCN:pH 5.0 buffer 45:55
Flow rate: 1
Detector: UV 210

REFERENCE

Roy,S.D.; Roos,E.; Sharma,K. Transdermal delivery of buprenorphine through cadaver skin, *J.Pharm.Sci.*, **1994**, *83*, 126–130.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: C8 Brownlee OSS Spheri-5
Column: 220 × 4.6 C8 Brownlee OSS Spheri-5
Mobile phase: MeCN:MeOH:10 mM pH 5 phosphate buffer 59.5:25.5:15
Flow rate: 1.5
Injection volume: 500
Detector: UV 215

OTHER SUBSTANCES
Also analyzed: prodrugs

REFERENCE
Stinchcomb,A.L.; Paliwal,A.; Dua,R.; Imoto,H.; Woodard,R.W.; Flynn,G.L. Permeation of buprenorphine and its 3-alkyl-ester prodrugs through human skin, *Pharm.Res.*, **1996**, *13*, 1519–1523.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 1.39

OTHER SUBSTANCES
Simultaneous: tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, nalorphine, phenazocine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: pemoline, benzphetamine, diethylpropion, mazindol, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene

REFERENCE
Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.3

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Inject an aliquot of a solution in MeOH.

HPLC VARIABLES
Guard column: present but not specified
Column: 250 × 4.6 OSS Spheri-5 C8
Mobile phase: MeCN:MeOH:pH 5 acetate buffer 25.5:59.5:15
Flow rate: 1.5
Detector: UV 285

OTHER SUBSTANCES
Simultaneous: prodrugs

REFERENCE
Stinchomb,A.L.; Dua,R.; Paliwal,A.; Woodard,R.W.; Flynn,G.L. A solubility and related physicochemical property comparison of buprenorphine and its 3-alkyl esters, *Pharm.Res.*, **1995**, *12*, 1526–1529.

SAMPLE
Matrix: tissue
Sample preparation: Extract skin with 10 mL MeCN by gentle agitation overnight. Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 220 × 4.6 5 μm Sheri-5 C8 (Brownlee)
Mobile phase: MeCN:MeOH:pH 5.0 phosphate buffer 60:25:15
Flow rate: 1.5
Injection volume: 20
Detector: UV 215

OTHER SUBSTANCES
Extracted: acetylbuprenorphine, butylbuprenorphine, isobutylbuprenorphine

KEY WORDS
mouse; skin

REFERENCE
Imoto,H.; Zhou,Z.; Stinchcomb,A.L.; Flynn,G.L. Transdermal prodrug concepts: Permeation of buprenorphine and its alkyl esters through hairless mouse skin and influence of vehicles, *Biol.Pharm.Bull.*, **1996**, *19*, 263–267.

SAMPLE
Matrix: urine
Sample preparation: Buffer the urine sample at pH 9.7. Extract with ethyl acetate:heptane (4:1). Dry the organic phase and reconstitute with mobile phase.

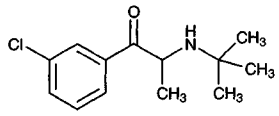
HPLC VARIABLES
Column: Hypersil silica
Mobile phase: MeCN:0.06% trifluoroacetic acid 90:10
Detector: MS, Sciex API III, heated nebulizer, positive ion mode

CHROMATOGRAM
Limit of quantitation: 0.2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, naloxone, norbuprenorphine

REFERENCE
Johnson,R.A.; Haan,D.E.; James,C.A.; Hopkins,N.K. Determination of linezolid, PNU-100766, in human plasma and urine using high-performance liquid chromatography with ultraviolet detection (Abstract 2487), *Pharm.Res.*, **1997**, *14*, S374–S374.

Bupropion



Molecular formula: $C_{13}H_{18}ClNO$
Molecular weight: 239.76
CAS Registry No.: 34911-55-2, 31677-93-7 (HCl)
Merck Index: 1523
Lednicer No.: 2 124

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 150 ng IS + 1 mL 600 mM pH 9.5 carbonate buffer + 10 mL n-heptane:isoamyl alcohol 98.5:1.5, shake for 15 min, centrifuge at 1500 g for 10 min. Remove the organic layer and add it to 250 μL 100 mM HCl, mix for 10 min, centrifuge at 1500 g for 10 min, inject an aliquot of the aqueous phase.

HPLC VARIABLES

Column: 250 × 4.6 5 μm LC-1 (Supelco)
Mobile phase: MeCN:buffer 20:80 (Buffer was 50 mM KH_2PO_4 containing 7 mM sodium heptanesulfonate and 10 mM triethylamine, adjust pH to 3.0 with phosphoric acid.)
Column temperature: 30
Flow rate: 2.3
Detector: UV 254 (UV 214 for metabolites)

CHROMATOGRAM

Retention time: 11.5
Internal standard: 2-(tert-butylamino)-4'-fluorovalerophenone hydrochloride (15.5)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: amitriptyline, amoxapine, chlorimipramine, chlorpromazine, desipramine, desmethyldoxepin, doxepin, fluphenazine, haloperidol, imipramine, loxapine, maprotiline, mianserin, nortriptyline, perphenazine, thioridazine, trazodone

KEY WORDS

plasma

REFERENCE

Cooper,T.B.; Suckow,R.F.; Glassman,A. Determination of bupropion and its major basic metabolites in plasma by liquid chromatography with dual-wavelength ultraviolet detection, *J.Pharm.Sci.*, **1984**, *73*, 1104–1107.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma or serum + 20 μL 1 μg/mL IS in 100 mM HCl + 400 μL 100 mM KOH, vortex for 5 s, add 5 mL hexane:isoamyl alcohol 96:4, vortex for 20 s, centrifuge at 2000 rpm for 6 min. Remove the organic layer and add it to 100 μL MeOH: 4 M HCl 99:1, evaporate to dryness under a stream of air at 30-35°, reconstitute the residue in 200 μL MeCN, inject a 60 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 3 μm Econosphere silica
Mobile phase: MeOH:buffer 95:5 (Buffer was 50 mM $(NH_4)H_2PO_4$ adjusted to pH 3.2 with 50 mM phosphoric acid.)

Flow rate: 0.9
Injection volume: 60
Detector: UV 248

CHROMATOGRAM

Retention time: 4.29
Internal standard: 2-(tert-butylamino)-4'-fluorobutyrophenone (234U66) (4.04)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Simultaneous: amitriptyline, amoxapine, benzphetamine, chlorpromazine, clozapine, desipramine, diethylpropion, diphenhydramine, haloperidol, imipramine, loxapine, nortriptyline, promethazine, propiomazine, sertraline, thioridazine, trazodone, trifluprom azine, trimeprazine
Noninterfering: chlorpheniramine, mesoridazine, thiothixene, trifluoperazine, trihexyphenidyl
Interfering: fluoxetine

KEY WORDS

plasma; serum; normal phase

REFERENCE

Jennison,T.A.; Brown,P.; Crossett,J.; Urry,F.M. A high-performance liquid chromatographic method for quantitating bupropion in human plasma or serum, *J.Anal.Toxicol.*, **1995**, *19*, 69–72.

SAMPLE

Matrix: blood, tissue
Sample preparation: Plasma. 1 mL Plasma + 500 μL saturated sodium borate, vortex briefly, add 5 mL MTBE, vortex briefly then mix on a reciprocating shaker for 10 min, centrifuge at 220 g for 10 min. Remove the organic phase and add it to 75 μL 10 mM phosphoric acid, vortex, centrifuge, inject a 50 μL aliquot of the aqueous layer. Tissue. Weigh whole brain and homogenize with 10 mL 340 mM perchloric acid containing 0.01 mM EDTA for 20 s (Brinkman PT 10/35). Remove a 1 mL aliquot and add 500 μL 600 mM sodium carbonate and 3 mL hexane:isoamyl alcohol 98:2 to it. Shake on a reciprocating shaker for 10 min, centrifuge at 220 g for 10 min, remove the organic layer and repeat the extraction. Combine the organic layers and add them to 75 μL 10 mM phosphoric acid, vortex, centrifuge, inject a 50 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 250 × 4.6 5 μm IBM reverse phase (trimethyl silane)
Mobile phase: MeCN:pH 3.0 phosphate buffer 27:73 containing 20 mM heptanesulfonic acid and 40 mM triethylamine
Flow rate: 1.5
Injection volume: 50
Detector: UV 214

CHROMATOGRAM

Retention time: 10.6
Internal standard: bupropion

OTHER SUBSTANCES

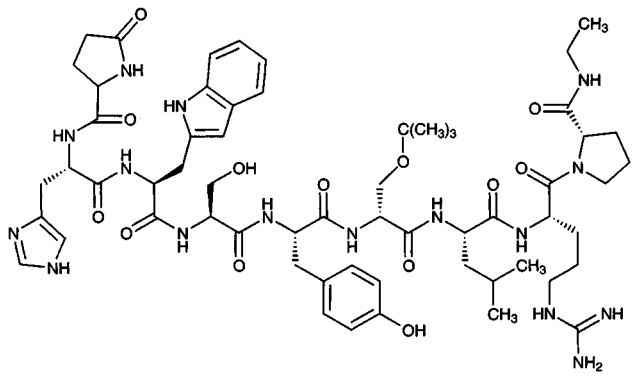
Simultaneous: trazodone

KEY WORDS
plasma; rat; brain; bupropion is IS

REFERENCE
Miller,R.L.; DeVane,C.L. Analysis of trazodone and m-chlorophenylpiperazine in plasma and brain tissue by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *374*, 388–393.

Buserelin

Molecular formula: $C_{60}H_{86}N_{16}O_3$
Molecular weight: 1239.44
CAS Registry No.: 57982-77-1, 68630-75-1 (acetate)
Merck Index: 1527



SAMPLE
Matrix: blood, urine
Sample preparation: Extract using a Sep-Pak C18 SPE cartridge.

HPLC VARIABLES
Column: 100 × 8 10 μm Bondapak Rad-Pak
Mobile phase: MeCN:120 mM pH 6.2 KH_2PO_4 65:35
Detector: radioimmunoassay

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; SPE

REFERENCE
Kiesel,L.; Sandow,J.; Bertges,K.; Jerabek-Sandow,G.; Trabant,H.; Runnebaum,B. Serum concentration and urinary excretion of the luteinizing hormone-releasing hormone agonist buserelin in patients with endometriosis, *J.Clin.Endocrinol.Metab.*, **1989**, *68*, 1167–1173.

SAMPLE
Matrix: cell incubations
Sample preparation: Inject a 60 μL aliquot.

HPLC VARIABLES
Guard column: 11 × 4 5 μm Nucleosil C18
Column: 250 × 4 5 μm Nucleosil C18
Mobile phase: Gradient. A was MeCN:water 0.5:99.5 containing 0.05% trifluoroacetic acid. B was MeCN:water 35:65 containing 0.05% trifluoroacetic acid. A:B 100:0 for 10 min, to 83:17 (step gradient), to 0:100 over 25 min.
Flow rate: 1
Injection volume: 60
Detector: UV 215 or F ex 280 em 365

CHROMATOGRAM
Retention time: 39

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
rat; kidney

REFERENCE
Kertscher,U.; Brudel,M.; Mehlis,B.; Sandow,J.; Berger,H. Pathways of degradation of buserelin by rat kidney membrane, *J.Pharmacol.Exp.Ther.*, **1995**, *273*, 709–715.

SAMPLE
Matrix: enzyme incubations
Sample preparation: Add 100 μL enzyme incubation (rat nasal mucosa homogenate) to 1 mL 100 mM HCl at 0°.

HPLC VARIABLES
Column: 150 × 6 Inertsil ODS-2
Mobile phase: MeCN:0.8% pH 6.2 KH_2PO_4 1:2
Flow rate: 1
Detector: F ex 280 em 350

KEY WORDS
rat

REFERENCE
Abe,K.; Irie,T.; Uekama,K. Enhanced nasal delivery of luteinizing hormone releasing hormone agonist buserelin by oleic acid solubilized and stabilized in hydroxypropyl-β-cyclodextrin, *Chem.Pharm.Bull.*, **1995**, *43*, 2232–2237.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4 5 μm Lichrosphere 100 RP-18
Mobile phase: MeCN:0.1% trifluoroacetic acid 23:77
Flow rate: 1
Detector: UV 214

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Hoitink,M.A.; Beijnen,J.H.; Boschma,M.U.S.; Bult,A.; Hop,E.; Nijholt,J.; Versluis,C.; Wiese,G.; Underberg,W.J.M. Identification of the degradation products of gonadorelin and three analogues in aqueous solution, *Anal.Chem.*, **1997**, *69*, 4972–4978.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 200 × 3 Spherisorb S5ODS-2
Mobile phase: Gradient. A was 0.05% phosphoric acid containing 0.5% $(NH_4)_2SO_4$. B was MeCN. A:B from 82:18 to 64:36 over 25 min, maintain at 64:36 for 2.5 min, return to initial conditions over 1 min, re-equilibrate for 6.5 min. or Isocratic MeCN:0.05% phosphoric acid containing 0.5% $(NH_4)_2SO_4$ 24:76
Flow rate: 0.5
Detector: UV 210

CHROMATOGRAM
Retention time: 22.5 (gradient), 20 (isocratic)

OTHER SUBSTANCES
Simultaneous: deslorelin, gonadorelin, goserelin, leuprolide, nafarelin

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Corran,P.H.; Sutcliffe,N. Identification of gonadorelin (LHRH) derivatives: comparison of reversed-phase high-performance liquid chromatography and micellar electrokinetic chromatography, *J.Chromatogr.*, **1993**, *636*, 87–94.

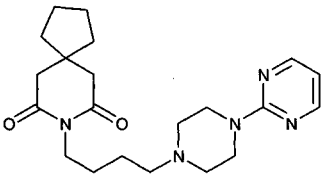
SAMPLE
Matrix: solutions
Sample preparation: 100 μL Incubation solution + 1 mL 100 mM HCl, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Nucleosil 100-5C18
Mobile phase: MeCN:100 mM phosphoric acid 2:7, adjusted to pH 2.5 with triethylamine

KEY WORDS
for buserelin acetate

REFERENCE
Matsubara,K.; Abe,K.; Irie,T.; Uekama,K. Improvement of nasal bioavailability of luteinizing hormone-releasing hormone agonist, buserelin, by cyclodextrin derivatives in rats, *J.Pharm.Sci.*, **1995**, *84*, 1295–1300.

Buspirone



Molecular formula: $C_{21}H_{31}N_5O_2$
Molecular weight: 385.51
CAS Registry No.: 36505-84-7, 33386-08-2 (HCl)
Merck Index: 1528
Lednicer No.: 2 300; 4 119

SAMPLE

Matrix: blood

Sample preparation: Condition a 100 mg Bond Elut C18 SPE cartridge with two 1 mL portions of MeOH and two 1 mL portions of 50 mM pH 7.2 KH_2PO_4 buffer. Centrifuge whole blood at 1500 g for 10 min. Add 50 µL 500 ng/mL prazosin to 1 mL plasma, vortex for 5 s, add 1 mL 50 mM pH 7.2 KH_2PO_4 buffer, vortex for 5 s, add to the SPE cartridge, dry in a stream of air, wash with two 1 mL portions of 50 mM pH 7.2 KH_2PO_4 buffer, wash with 500 µL MeOH. Dry the cartridge in a stream of air, let stand for 15 min, elute with 1 mL MeCN:25% ammonium hydroxide 99:1, evaporate the eluate to dryness under a stream of nitrogen, dissolve the residue in 200 µL mobile phase, inject an aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 Supelguard ABZ+plus C18 (Supelco)
Column: 250 × 4.6 Supelcosil ABZ+plus C18 (Supelco)
Mobile phase: MeCN:50 mM pH 6.5 KH_2PO_4 buffer 30:70
Flow rate: 1
Detector: E, ESA Coulochem II, 5011 model analytical cell, guard cell +950 mV, first electrode +600 mV, second electrode +900 mV

CHROMATOGRAM

Retention time: 11.24
Internal standard: prazosin (6.14)
Limit of detection: 60 pg/mL
Limit of quantitation: 100 pg/mL

KEY WORDS

plasma; SPE

REFERENCE

Ary,K.; Róna,K.; Ondi,S.; Gachályi,B. High-performance liquid chromatographic method with coulometric detection for the determination of buspirone in human plasma by means of a column-switching technique, *J.Chromatogr.A*, **1998**, *797*, 221–226.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 236.9

CHROMATOGRAM
Retention time: 12.523

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: dialysate
Sample preparation: Inject an aliquot directly.

HPLC VARIABLES
Column: 150 × 1 5 μm Sepstik CN-5μ (Bioanalytical Systems)
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 15:85:0.1, adjusted to pH 3.0 with orthophosphoric acid
Flow rate: 0.06
Injection volume: 10
Detector: E, BAS 4C, glassy carbon working electrode +1.10 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 6.2
Limit of detection: 1 ng/mL
Limit of quantitation: 10 ng/mL

KEY WORDS
microbore; rat; brain; pharmacokinetics

REFERENCE
Tsai,T.H.; Chen,C.F. Measurement and pharmacokinetic analysis of buspirone by means of brain microdialysis coupled to high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1997**, *762*, 269–273.